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Prevalence of Antibodies to Hepatitis E Virus Among Hemodialysis Patients in Sweden

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In order to study the prevalence of antibody to hepatitis E virus (HEV) among hemodialysis patients and to evaluate whether chronic hemodialysis is associated with an increased risk of exposure to HEV in developed countries, the IgG anti-HEV was determined in serum samples obtained from 182 patients on chronic hemodialysis and 349 statistically selected, healthy Swedish control subjects. Serum specimens from 11 of the 182 (6.0%) hemodialysis patients and from 18 of the 349 (5.2%) control subjects were repeatedly positive for HEV antibodies (the difference was not significant: $P = .67$). Analysis of serial serum samples obtained at the initiation of hemodialysis and consecutively during follow-up periods of several years demonstrated no IgG anti-HEV seroconversion during chronic hemodialysis. The seroprevalence of anti-HEV antibody in the adult Swedish population was associated significantly with age. In persons younger than 40 years, the percentage of seropositive individuals was 2.5%, whereas the seroprevalence rate of anti-HEV was 7.4% in subjects older than 40 years ($P < .05$). This study indicates that nosocomial transmission of HEV to patients on maintenance hemodialysis was non-existent in three dialysis centers in Sweden (a developed country) and that chronic hemodialysis is not associated with an increased risk of exposure to HEV infection in this region. ***J. Med. Virol. 54:38–43, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: hemodialysis; IgG anti-HEV; Sweden; developed countries

INTRODUCTION

Hepatitis E virus (HEV) is the causative agent of the enterically transmitted, non-A, non-B hepatitis (ET-NANBH) [Reyes et al., 1990; Tam et al., 1991]. Epidemics of ET-NANBH have been documented in tropical and subtropical regions, where they can generally be traced to fecal contamination of drinking water [Viswanathan, 1995]. HEV may be a zoonotic virus of swine [Clayson et al., 1995], and non-human primates [Arankalle et al., 1994] and rats [Karetnyi et al., 1993; Maneerat et al., 1996] have been suggested to be among its natural hosts. Seroprevalence studies of anti-HEV in endemic and nonendemic regions of the world have revealed a close correlation between the level of sanitation, the incidence of disease, and the prevalence of anti-HEV [Thomas et al., 1993].

In industrialized countries, it is generally believed that only international travelers face the risk of HEV infection [Skidmore, 1995]. Recently, solid-phase enzyme immunoassays with recombinant antigens or synthetic peptides have been introduced for the diagnosis of this infection, and forthcoming data suggest that HEV infection may be more common outside developing countries than was previously anticipated [Zanetti et al., 1994; Coursaget et al., 1994; Zaaijer et al., 1995; Quiroga et al., 1996; Psichogiou et al., 1996a,b]. A 1–3% seroprevalence of HEV antibodies among volunteer blood donors and pregnant women has been reported from several western central European countries and the United States. Recent findings in Germany [Langer and Frösner, 1996], the Netherlands [Zaaijar et al., 1995], Italy [Zanetti et al., 1994], Greece [Psichogiou et al., 1996a,b], and Sweden [Johansson et al., 1995] suggest that absence of travel to an endemic region does not necessarily exclude the diagnosis of acute hepatitis E infection.

Although transmission is generally via the feco-oral route, intravenous drug abuse [Lavanchy et al., 1994] has been suggested as a risk factor associated with the presence of anti-HEV in nonendemic countries. One study has shown a striking association between hepatitis C virus (HCV) and HEV, pointing to similar or overlapping routes of transmission [Pisanti et al., 1994]. Patients undergoing chronic hemodialysis have an increased risk of exposure to nosocomially transmit-

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TABLE I. Hepatitis E Virus (HEV) Reactivity in Hemodialysis Patients

Disease (no. of patients)	HEV-reactivity[a]	No	[%]	Sex F/M	Median Age, years (range)
Hemodialysis	Anti-HEV pos.	11	[6]	3/8	67 (46–90)
(n = 182)	Anti-HEV neg.	171	[94]	70/101	64 (20–90)
Control group	Anti-HEV pos.	18	[5]	11/7	53 (27–90)
(n = 349)	Anti-HEV neg.	331	[95]	176/155	47 (20–91)

[a]HEV-reactivity assessed by Abbott HEV-EIA.

ted agents, and the possibility of transmission of HEV in this group of patients had been raised [Halfon et al., 1994].

The aim of the study was to establish the prevalence of anti-HEV among hemodialysis patients in a nonendemic country like Sweden and to evaluate whether chronic hemodialysis is associated with an increased risk of exposure to HEV, compared with a statistically selected, normal Swedish population.

PATIENTS AND METHODS

Patients

The study group consisted of hemodialysis patients followed up from the years 1990–1995 at three different hemodialysis units in Stockholm, Sweden. Hemodialysis serum samples for screening were collected during 1994. In order to determine the performance of the test for antibody to HEV for the diagnosis of hepatitis E in settings with a low incidence of acute hepatitis E, a representative cohort of 349 adult, healthy Swedish volunteers was selected from a random sample of 4,800 persons. First, parishes were randomly selected, and the number of parishes was correlated to the population in the area. In each parish, 24 men and 24 women aged 18–90 years were selected. They were contacted and offered an investigation of immunity to poliomyelitis, diphtheria, and tetanus. Those who were not protected satisfactorily were offered appropriate vaccination. They also agreed that their blood samples could be used for other investigations, except HIV tests. These further tests would be carried out anonymously. The main collection was made in 1991. Of the 4,800 persons contacted, 3,390 agreed to participate (71%). The number of tested individuals per age group was closely correlated to the general, healthy Swedish population. The study was approved by the Ethics Committee of the Karolinska Institute [Böttiger et al., 1997].

Hemodialysis patients reactive to the anti-HEV test were studied further in relation to the clinical and epidemiological findings, as the purpose of the study was to determine whether the presence of anti-HEV reactivity reflected HEV infection acquired during chronic hemodialysis and whether anti-HEV reactivity reflected a past HEV infection contracted in an HEV-endemic country or not.

Methods

Frozen sera, stored at −20°C, were tested retrospectively for anti-HEV IgG, using diagnostic kits (Abbott GmbH Diagnostica, Wiesbaden-Delkenheim, Germany). This assay included two recombinant HEV antigens, SG-3, which consisted of 327 amino acids from the carboxyl half of ORF 2 encoding the major structural proteins of HEV, and 8-5, which consisted of 123 amino acids representing the full-length ORF3 obtained from the Burmese strain of HEV [Dawson et al., 1992]. The proteins were co-coated on polystyrene beads to serve as the antigenic target for EIAs. The serum samples were assayed for anti-HEV reactivity according to the manufacturer's instructions. The absorbance for sample (S) over Cut-Off (CO) was calculated according to the manual. A sample with a S/CO value >1 was considered initially reactive, and the calculation was then repeated in duplicate or triplicate. Because of the lack of a commercially available, confirmatory assay, all the subsequent serum samples that were obtainable from the reactive hemodialysis patients were tested for IgG anti-HEV in a similar way. An individual was considered to be seropositive if anti-HEV IgG was detected in all subsequent samples [Balayan et al., 1996]. This approach was applied to all hemodialysis patients, but not to the statistically selected, normal Swedish population.

Statistical Analysis

The level of significance (*P* values) was obtained, using the unpaired Student *t*-test.

RESULTS

A commercially available HEV enzyme immunoassay (EIA) was used for the detection antibody to HEV. To establish the seroprevalence rate of anti-HEV antibodies among hemodialysis patients and healthy adult Swedish residents, serum specimens drawn from 182 hemodialysis patients and 349 healthy individuals were analyzed for IgG anti-HEV reactivity. Anti-HEV reactivity with S/CO > 1 was detected in 11/182 (6.0%) of the hemodialysis patients and in 18/349 (5.2%) of the statistically selected, control population (Table I).

The distribution of S/CO values for the hemodialysis patients was not significantly different when compared with that of the reference group ($P = .67$) (Fig. 1). The participants in the two populations were divided into only two age groups (i.e., those aged 40 years or younger and those aged 41 years or older). The prevalence of antibodies in the two groups is presented in Table II. The anti-HEV distribution was age dependent among the 349 healthy individuals in the Swedish reference population, ranging from 2.5% (4/159) in sub-

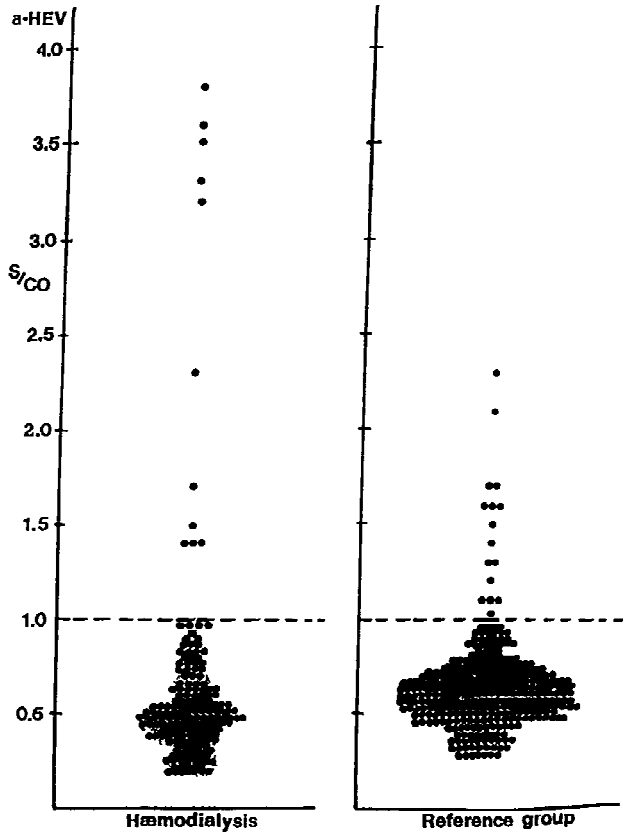

Fig. 1. Anti-HEV reactivity in sera from hemodialysis patients compared with a reference population in Sweden. Serum samples from 182 patients in chronic hemodialysis and 349 healthy adult individuals (see Patients and Methods section) were analyzed for anti-HEV reactivity by Abbott HEV-EIA by standard procedure. Data are given as sample/cut-off (S/CO).

TABLE II. Percentage of Hepatitis E Antibody-Positive Persons in Two Age Groups in a Swedish Reference Population and in Hemodialysis Patients*

	Age group (20–40 years)		Age group (41–91 years)		
Study population	No.	% HEV pos.	No.	% HEV neg.	Statistics
Hemodialysis patients	20	0	162	6.8	NS
Control group	159	2.5	190	7.4	$P < .05$

*HEV reactivity assessed by Abbott HEV-EIA. NS, not significant.

jects aged 40 years or younger to 7.4% (14/190) in subjects aged 41 years or older ($P < .05$) (Table II; Fig. 2b). None of the hemodialysis patients under 40 years of age were positive for the antibody, whereas 6.8% of the older age group were positive (Table II). This difference, however, was not significant. Subjects demonstrating anti-HEV reactivity with S/CO > 2.0 were aged 50 years or older (Fig. 2a,b).

The details of age, sex, anti-HEV IgG reactivity, and country of origin for the HEV-seropositive hemodialysis patients are shown in Table III. Two of the five patients who exhibited high anti-HEV IgG reactivity

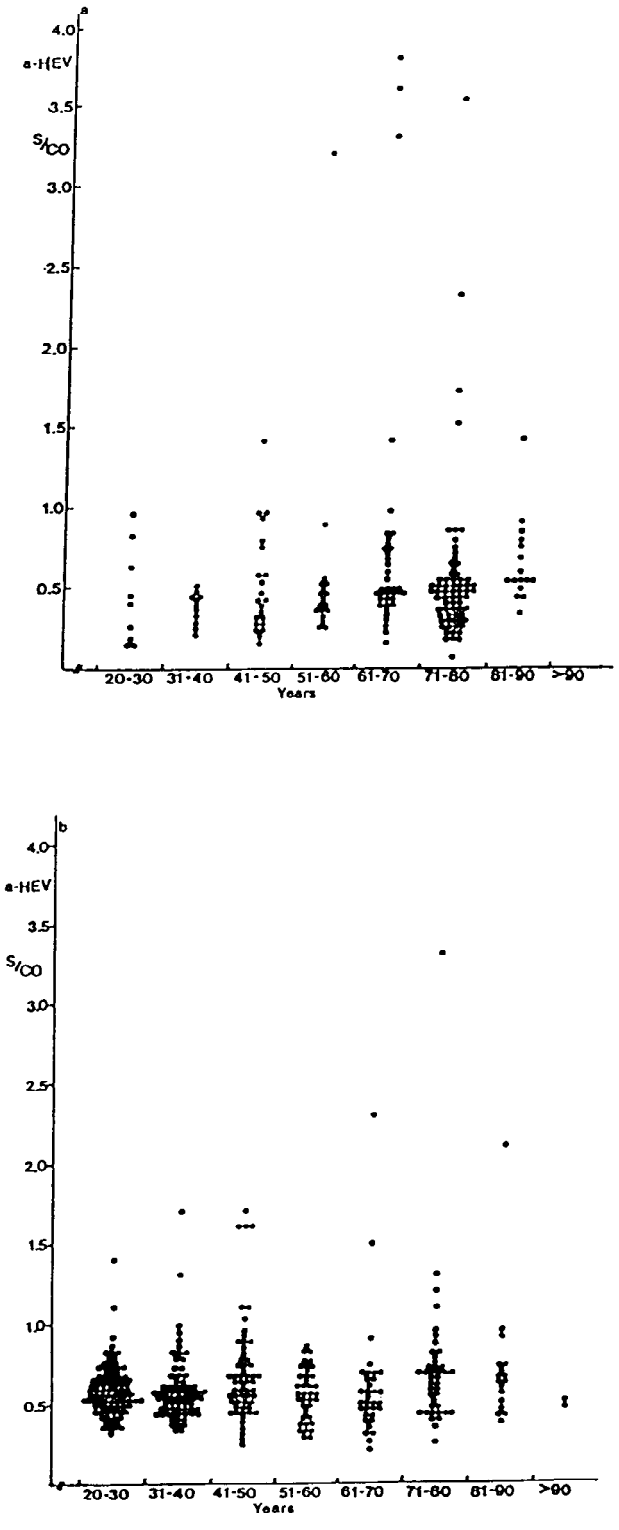

Fig. 2. **a:** The age-specific prevalence of antibody to hepatitis E virus in hemodialysis patients. Data obtained by Abbott HEV-EIA on sera collected in 1994. **b:** The age-specific prevalence of antibody to hepatitis E virus in the control population. Data obtained by Abbott HEV-EIA on sera collected in 1991.

TABLE III. Age, Sex, Anti-HEV Reactivity, and Country of Origin in Patients on Chronic Hemodialysis With Antibodies Against HEV

Patient (No.)	Age (years)	Sex	Anti-HEV S/CO[a]	Country of origin
1	65	M	3.8	India
2	65	M	3.6	Italy
3	72	M	3.5	France
4	62	M	3.3	Iran
5	53	F	3.2	Sweden
6	73	M	2.3	Sweden
7	76	M	1.7	Sweden
8	80	F	1.5	Sweden
9	46	M	1.4	Sweden
10	90	M	1.4	Sweden
11	59	F	1.4	Germany

[a]Anti-HEV reactivity as assessed by Abbott HEV-EIA. S/CO, sample/cut-off.

(e.g., S/CO > 3.0) were from countries where hepatitis E is endemic, namely, India and Iran. One patient was born and had grown up in Italy and another was from France. The remaining patient was a Swedish woman with no epidemiological data, and therefore previous visits to HEV-endemic regions could not be completely ruled out.

It is noteworthy that the individual with the highest anti-HEV reactivity, a 65-year-old male, had immigrated to Sweden from India 6 years earlier. In 1984, he had been hospitalized in India because of acute clinical hepatitis of unknown origin. He had serological markers of past hepatitis A virus infection but was seronegative for markers of hepatitis B and C. It was judged that his high level of anti-HEV was a reflection of previous exposure to HEV, and possibly a consequence of acute hepatitis E contracted 10 years previously in his native country.

The remaining anti-HEV reactive patients did not report any history of clinical viral hepatitis, travel abroad, or contact with persons coming from HEV-endemic parts of the world. Due to the anonymous testing for anti-HEV reactivity in the control subjects, no epidemiological data were available for those who were anti-HEV positive.

In order to establish whether seroconversion to anti-HEV positivity had occurred during the period of chronic hemodialysis, IgG anti-HEV patterns were determined in the first available serum sample from each anti-HEV reactive patient before entering into hemodialysis and in consecutively obtained serum samples from each patient during several years of chronic hemodialysis. IgG anti-HEV was detected at the initiation of hemodialysis and persisted for several years, in some patients at a diminished level, through the follow-up period, demonstrating that none of the hemodialysis patients had seroconverted to anti-HEV reactivity during the period of chronic hemodialysis (data not shown).

DISCUSSION

Several studies have demonstrated recently the usefulness of serological tests, based on the use of recombinant antigens, for the detection of antibodies to HEV in the diagnosis of acute HEV hepatitis in epidemic and sporadic settings [Yarbough et al., 1991; Favorov et al., 1992; Dawson et al., 1992; Goldsmith et al., 1992]. Recombinant antigen-based assays have also been introduced for serological surveillance studies in selected groups of clinical patients and healthy subjects living in endemic areas [Lok et al., 1992; Balayan et al., 1994]. There are limited data on the utility of these assays for determining the epidemiology of HEV in hemodialysis patients and among healthy people in non-endemic countries. We found that the prevalence of anti-HEV IgG was 6.0% in chronic hemodialysis patients attending three different units in Stockholm and was not significantly different from the 5.2% seroprevalence exhibited by the healthy adult Swedish reference group. None of the 182 patients who underwent chronic hemodialysis seroconverted to anti-HEV during an observation period of several years.

Taken together, these results suggest that chronic hemodialysis in Sweden, where HEV is not endemic, has not been associated with an increased risk of exposure to HEV, and nosocomial transmission in this particular population from Stockholm County could not be demonstrated.

Similar findings have been reported from Spain, where 5.5% of healthy adults and 6% of patients on hemodialysis were detected and confirmed as reactive for IgG anti-HEV antibodies by a synthetic-peptide-based EIA [Buti et al., 1995]. Moreover, recent studies have indicated that HEV is probably not transmitted during chronic hemodialysis in France [Halfon et al., 1996], Greece [Psichogiou et al., 1996a,b], and Italy [Gessoni and Manoni, 1996; Fabrizi et al., 1997], and that the early reports of high prevalences of anti-HEV IgG in hemodialysis patients in uncontrolled studies were possibly due to the confounding effect of age.

It is now well recognized by epidemiological analysis of populations with high prevalences of anti-HEV in India [Arankalle et al., 1995], Turkey [Thomas et al., 1993], Southern Italy [Zanetti et al., 1994], the former Soviet Union [Balayan et al., 1994], and the United States [Purcell and Tsarev, 1996] that seroprevalence rises in the late teens and early adulthood, at which time the peak incidence of HEV infection is likely to occur. An age-dependent increase in seropositivity was also observed in our reference population corroborating the generality of this finding. However, the pattern of age-specific seroprevalence differed from those reported from endemic areas, increasing before the fourth decade of life rather than in the fifth, as we observed. Our findings closely parallel that in a community-based sample in inner-city London [Bernal et al., 1996], where an overall 5.3% anti-HEV seroprevalence was found, suggesting either that infection occurs in an older age group in the United Kingdom and Sweden or, more likely, that the increased prevalence later in life reflects an accumulation of anti-HEV reactivity in a cohort of subjects infected in the past. Similarly, high levels of seroprevalence for anti-HAV and anti-

HBV antibodies in the oldest age groups of the Swedish population have recently been reported [Böttiger et al., 1997; Christenson et al., 1997]. From our study it is impossible to be certain whether the infection of anti–HEV-positive subjects occurred in Sweden or abroad, as no detailed travel history was available.

The 13 hemodialysis patients with IgG anti-HEV antibody at the initiation of chronic hemodialysis remained reactive to the HEV EIA without waning anti-HEV levels during a follow-up period of several years. The duration of anti-HEV IgG in reconvalescents remains to be definitively determined. HEV antibodies have been shown to persist for at least 14 years after the acute disease in some adult patients [Khuroo et al., 1993], whereas in children anti-HEV IgG disappears or sharply declines within a year after the onset of disease [Goldsmith et al., 1992]. Interestingly, one of the adult hemodialysis patients had high titers of anti-HEV when tested 10 years after acute ET-NANB hepatitis contracted in India (e.g., S/CO > 3.0).

The prevalence of anti-HEV antibodies in the chronic hemodialysis patients, as well as in the Swedish control population, was higher than expected for a lowly endemic country. The results of several studies showed that the prevalence of antibody to HEV in regions of non-endemicity was higher than expected, whereas the prevalence of anti-HEV in highly endemic areas was lower than anticipated [Dawson et al., 1992; Paul et al., 1994].

In our study, however, the level of anti-HEV reactivity was greater than S/CO values of 3.0 in some of the hemodialysis patients, but in the majority of cases in the reference group it was only elevated marginally (e.g., S/CO < 2 > 1). The significance of slightly raised anti-HEV values, when assayed by the commercially available test in low-risk populations, in which virtually no cases of clinical HEV disease are observed, is not clear. In view of the absence of a reported history of jaundice in the majority of anti–HEV-reactive persons, anti-HEV reactivity at low levels has been suggested to be a consequence of unapparent infections [Arankalle et al., 1995], perhaps by low-viral-dose infection or with attenuated strains of HEV [Psichogiou et al., 1996a], possibly from an animal reservoir. Alternatively, it cannot be excluded that other related viruses may share antigens with HEV and cross-react in the anti-HEV EIA [Koonin et al., 1992], or that contaminating *Escherichia coli* antigens produced by recombinant techniques may interfere with the specificity of the assay.

Further studies using supplemental tests to improve the predictive value of anti-HEV screening may help to clarify these possibilities. In the meantime, prudence is warranted in the interpretation of seroprevalence data, especially in regions of low endemicity, where only a small proportion of those with anti-HEV have a history of jaundice or a detailed travel history to a region in which HEV is endemic.

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